

YKL-40 IN HEALTH AND DISEASE: A CHALLENGE FOR JOINT INFLAMMATION

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*There is a need of biomarkers to detect early joint inflammation and destruction of cartilage in different types of arthritis. YKL-40, a 39 kD heparin- and chitin-binding secreted glycoprotein (also known as cartilage gp39), was recently discovered. Its exact biological function is still unclear. Specific receptors for YKL-40 have not been identified yet. The clinical significance of YKL-40 as a biomarker is discussed in different aspects. High level of YKL-40 was found in various human diseases associated with inflammatory and neoplastic processes. The review highlights the information available about YKL-40 and its significance in inflammatory joint diseases. We suggest that this glycoprotein might have a promising value as a novel biomarker and could provide additional evidence for inflammation activity in different types of arthritis. **Biomed Rev 2013, 24: 49-56***

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INTRODUCTION

Rheumatic joint diseases are common disorders that affect a large part of the human population. They are associated with progressive disability, early death and socioeconomic costs (1). Some of them are manifested as acute or degenerative inflammatory arthritis. Almost all types of arthritis are characterized by destruction of articular tissue, synovial inflammation and angiogenesis, bone and cartilage loss (2).

Lots of research efforts are made to identify a biomarker, which alone or in combination with other conventional markers could be useful for diagnosis and monitoring of these diseases.

YKL-40, a 39 kD heparin- and chitin-binding glycoprotein, is a novel potential biomarker of inflammatory processes, which is expressed and secreted by immune cells such as activated macrophages and neutrophils (3). Several studies indicate that secretion levels of this glycoprotein are elevated in rheumatoid arthritis (4), osteoarthritis (5), heart failure (6), asthma and chronic obstructive pulmonary diseases (7). In addition, independent investigations reveal that high serum YKL-40 levels are correlated with aggressiveness and poor survival in different malignancies such as glioblastoma (8),

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ovarian cancer (9), breast and colorectal cancer (10,11). Despite of these evidences some authors disagree with the significance of YKL-40 in inflammatory and neoplastic processes (12).

The present review focuses on the importance of YKL-40 in inflammatory joint diseases and on its value as a diagnostic or prognostic marker for disease activity.

YKL-40: GENE AND PROTEIN

YKL-40 contains a single polypeptide chain, comprising of 383 amino acids and has a molecular mass of 40 kD (13,14). Amino acid sequencing showed that it belongs to the glycosyl hydrolase family 18 (15). This family includes enzymes and proteins, chitinases from different species (mammalian, bacteria, fungi, nematodes, insects and plants) (16). All “mammalian chitinase-like proteins” show a high level of sequence identity over certain regions. The N-terminal amino acid sequence and the catalytic center are highly conserved (>70% identical), whereas the identities are low in the C-terminal sequence. It has also been demonstrated that *Drosophila melanogaster* secretes several proteins with sequence identity to YKL-40 (16-34%). The nematode *Caenorhabditis elegans* and the zebra fish *Danio rerio* have multiple putative YKL-40-like proteins (18%-30%) with sequence identity (3).

Two mutations of the catalytic glutamic and aspartic acids to leucine and to alanine respectively are responsible for the lack of hydrolase activity of YKL-40 (17). The human YKL-40 gene is located on chromosome 1q32.1 and consists of 10 exons (18). A study on the transcriptional regulation of YKL-40 during human macrophage differentiation suggests that the YKL-40 gene in monocytes is inactivated and requires additional events to initiate promoter activity. The promoter sequence contains binding sites for several known factors. The Sp1-family transcription factor has a major role in controlling YKL-40 promoter activity (18).

The crystallographic structure of human YKL-40 exhibits two globular domains, which form a groove and correspond to the active site of the protein (15).

YKL-40: REGULATION AND BIOLOGICAL FUNCTION

Few data is available about the regulation of YKL-40, and these are quite contradictory. YKL-40 secretion in freshly isolated chondrocytes is stimulated by interleukin (IL-6, IL-17, IL-18) and tumor necrosis factor-alpha (TNF- α) (19-21). Insulin-growth factor-I (IGF-I) and insulin-growth factor-II (IGF-II) induce YKL-40 secretion in guinea pig chondrocytes

but not in human chondrocytes (20,22). Most probably, the results might be due to differences in the investigated species. Some authors determined that hypoxia and ionizing radiation activated YKL-40 secretion in glioblastoma cell lines - U87, U118, U373, while fibroblast growth factor (FGF) and TNF- α suppressed the glycoprotein production (23).

Zhang *et al* revealed that resveratrol inhibited YKL-40 expression by influencing its promoter activity and mRNA transcription levels in U87 cells in *in vitro* conditions (24). Other researchers showed that IL-1 β was the potential inducer of astrocytic YKL-40 transcription. The proinflammatory interleukins such as IL-2, IL-6, IL-12, IL-13, IL-17 and IL-18 did not induce YKL-40 transcription in astrocytes (25). The summarized data about known inducers and suppressors of YKL-40 secretion is presented in Figure 1.

These contradictory results emphasize the differences in the *in vitro* and *in vivo* effects of YKL-40 on cellular and systemic response. We can assume that YKL-40 might play various roles depending on the cell type. Changes in the extracellular matrix also affect YKL-40 synthesis. Microarray gene expression analysis determined that YKL-40 was overexpressed 4.4 fold in dedifferentiated human fetal chondrocytes in comparison with differentiated chondrocytes (26).

The level of YKL-40 secreted by normal cartilage explants is low during the first days of culture but after a few days increases significantly. YKL-40 production is also induced by cartilage resection or by removal of chondrocytes from their native environment (20).

A study on the expression of YKL-40 in mouse mammary tissue found that YKL-40 was expressed feebly prior to and during pregnancy and lactation and increased during mammary gland involution, which is characterized by intensive tissue remodeling (27).

The exact function of YKL-40 continues to be unknown and specific receptor for YKL-40 has not been yet identified. It has been suggested that it probably plays a role in cell proliferation and differentiation (28). Other researchers revealed that YKL-40 ensured protection against apoptosis during mammary involution (29) or preserved the extracellular matrix during tissue remodeling *via* suppression of different types of matrix metalloproteinases (30).

YKL-40 was supposed to induce signaling pathways in connective tissue and thus acts as a growth factor for fibroblasts, synovial cells and chondrocytes (28). Several studies showed a strong relationship between YKL-40 and vascular cells. YKL-40 functions as a migration and adhesion factor for these cells and activates formation of branching

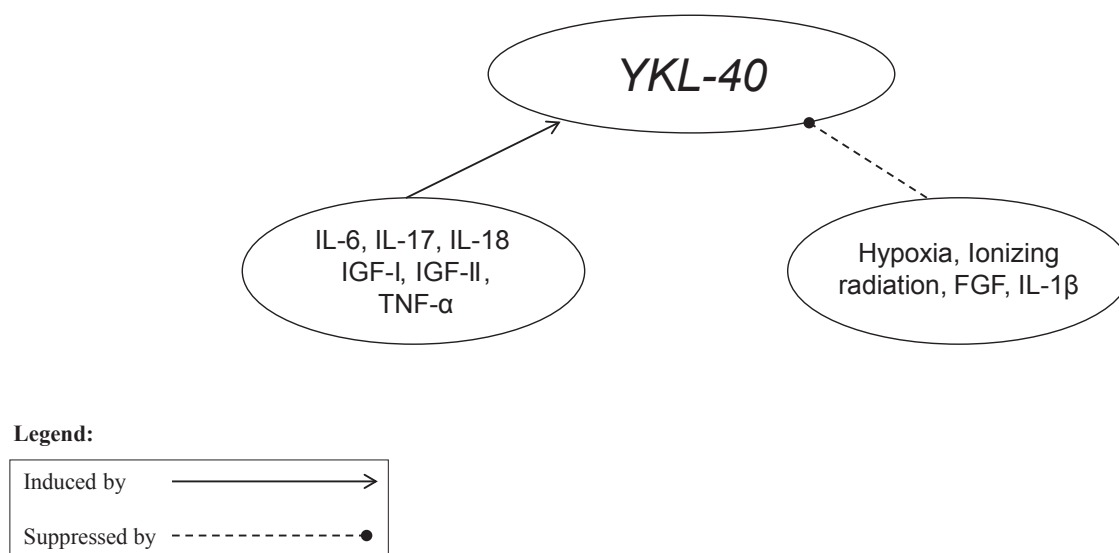


Figure 1. Inducers and inhibitors of YKL-40 secretions in vitro.

tubules, hence could play a role in angiogenesis (31).

There is growing body of evidence that increased expression and secretion of YKL-40 are associated with the pathogenesis of multiple human diseases.

YKL-40 IN NORMAL CONDITIONS

Cell expression of YKL-40

Intensive expression of mRNA^{YKL-40} is observed in all germ layers of human embryos, which are characterized with rapid proliferation and differentiation. YKL-40 protein is present in high concentrations during development of cartilage, bone, joints, and muscles (32). In normal adult human tissue, YKL-40 is intensely expressed in mast cells, polymorphonuclear granulocytes, basal epithelial cells and neurons (33).

Serum levels of YKL-40

There is no fixed reference value for YKL-40 in healthy persons. It has been determined that the level of the glycoprotein increased with age and it is essential to use an identical age-matched control group (34). Johansen suggested that changes in serum YKL-40 concentration higher than 20% should be considered as elevated serum values (3). It was determined that serum YKL-40 was stable in healthy subjects for short-term as well as for long-term sampling periods of up to 3 years (35).

Recently published levels of YKL-40 in healthy people

differ among populations. The median serum concentration of the protein is around 43 ng/ml in Danish healthy individuals (35), while in the Turkish and Japanese population it is higher (5,36). This difference could be explained with the fact that some studies use serum and other plasma samples. Various immunoassays (ELISA, RIA) also could give divergent YKL-40 levels.

YKL-40 is not investigated in detail in Bulgaria yet. Our research is the first to reveal data for circulating YKL-40 in healthy Bulgarian subjects. We determined that the median serum value of YKL-40 in 40 healthy people, aged 53.69 ± 3.30 , was 84.19 ± 11.39 ng/ml (37). The comparative data about serum YKL-40 concentrations in different healthy populations is included in Table 1.

YKL-40 IN JOINT DISEASES

It is hypothesized that YKL-40 participates in acute and chronic inflammation, based on the fact that this glycoprotein is over-expressed in pathological conditions associated with active inflammatory processes.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology characterized by symmetrical synovial inflammation of the joints (41). The identified genetic predis-

Table 1. Serum YKL-40 levels (ng/ml) in different healthy populations

Nº	Population	Serum YKL-40 levels	References Nº
1	Danish	43	35
2	French	59	38
3	Chinese	61.1	39
4	Bulgarian	84.19	37
5	Japanese	101.7	40
6	Turkish	114	5

position as risk factors for RA is the linkage to MHC class II antigens HLA-DRB1 404 and HLA-DRB1 401 (42). It is discovered that YKL-40 contains HLA-DR4 binding motifs, so it is supposed to act as an autoantigen in RA (43).

Reliable biomarkers of joint inflammation and destruction in RA patients are proteins released by cells in synovial fluid and serum. Some researchers have observed a 10-fold increase in the concentration of YKL-40 in synovial fluid in comparison with serum levels in RA patients. A significant relation between glycoprotein levels in serum and synovial fluid was detected in different populations with RA (44,45). It is suggested that YKL-40 in synovial fluid and serum might reflect cartilage degradation and synovial inflammation in RA (3,46).

The data about the importance of serum YKL-40 as a novel biomarker for the risk of progression of joint damage in patients with RA is contradictory. Some authors assume that it could be useful as a serious parameter in disease diagnosis and monitoring (36), other disagree with this statement (47). As mentioned above, YKL-40 is implicated in the process of angiogenesis (31), which is also essential in nourishing synovial tissue and has a central role in synovial inflammation and pannus formation (48).

Recent immunohistochemical study found that YKL-40 was expressed by polymorphonuclear cells in the synovial fluid of RA patients (48). This observation might provide another view on the inflammatory process and disease activity in comparison with conventional parameters. In this context, we suppose that YKL-40 may stimulate angiogenesis or acts as growth factor for chondrocytes and/or synovial fibroblasts. The levels of YKL-40 in inflammatory joint arthritis are presented in Table 2.

Our investigation of RA patients showed a significant elevation of serum and synovial YKL-40 levels (48), strongly

correlated with conventional parameters such as C-reactive protein and erythrocyte sedimentation rate. We determined that YKL-40 level in synovial fluid was significantly higher compared to the serum level and there was a positive correlation between them. We used sonography as a technique more sensitive for detecting lesions than conventional radiography. This is the first study that demonstrated a relationship between YKL-40 levels and ultrasonographic examinations. We suggested that the increased concentration in serum and synovial fluid of YKL-40 may indicate destructive changes in the cartilage (37).

Osteoarthritis

Degraded cartilage and inflamed or thickened synovial tissue are the major manifestations of osteoarthritis (OA) (56). Several studies determined increased serum and synovial YKL-40 levels in patients with severe OA compared to healthy subjects (5,48). Zivanovic *et al* also suggested that YKL-40 could be used as a biomarker of joint damage in knee osteoarthritis (50). Other researchers did not detect YKL-40 mRNA expression in normal human cartilage chondrocytes, while there were increased levels of the glycoprotein in articular cartilage from OA patients (51). High serum and synovial YKL-40 concentrations might reflect joint degradation and synovial inflammation (3). An association between the number of YKL-40 positive cells and the severity of the synovitis was also observed (36).

It was demonstrated that YKL-40 bound to partially acetylated chito-oligosaccharides acted as a growth factor for chondrocytes in culture (57). They revealed that these biomaterials could benefit the symptoms of inflammatory joint disorders more effectively than glucosamine. Likewise, YKL-40 itself may be a target for a novel therapeutic approach in OA (57).

Psoriatic and gout arthritis

Psoriatic and gout arthritis are chronic inflammatory disorders, which have different etiology but manifest common clinical features. The pathogenesis of these diseases is still incompletely revealed. The pathophysiological role of synovium and the regulation of cytokine biosynthesis are just beginning to be elucidated (58,59). There are only two small scale studies on YKL-40 in psoriatic arthritis, which determine significantly higher serum YKL-40 levels in patients compared to healthy people (52,53).

Our preliminary results show that in all patients with psoriatic and gout arthritis the concentration of serum YKL-

Table 2. Serum and synovial YKL-40 (ng/ml) levels in patients with inflammatory joint diseases

Nº	Diagnosis	Number of patients	YKL-40 in serum	YKL-40 in synovial fluid	References Nº
1	Knee osteoarthritis	55	131.35 ± 90.91	-	5
2	Knee osteoarthritis	88	138.22 ± 48.88	-	50
3	Knee osteoarthritis	39	73 (26-565)	119 (274-2600)	51
4	Hip osteoarthritis	45	67.5 (33-343)	-	38
5	Rheumatoid arthritis	308	86	-	4
6	Rheumatoid arthritis	40	246.18 ± 209.36	509.77 ± 375.56	37
7	Rheumatoid arthritis	20	87 (20-218)	212 (485-6850)	51
8	Spondyloarthritis	49	74 (14-572)	-	52
9	Psoriatic arthritis	42	112		53
10	Psoriatic arthritis	14	128.06	353.6	54
11	Gout arthritis	7	277.27 ± 126.95	769.61 ± 61.02	55

40 is remarkably elevated. The synovial level of the protein is significantly higher in comparison with the serum value. No evident relationship between the concentration of YKL-40 and proinflammatory cytokines in patients with psoriatic and gout arthritis was established. Whereas a strong association between serum and synovial levels of YKL-40 and serum TNF- α and IL-1 β in patients with RA patients was detected. We suggest that the different concentration of YKL-40 in the three types of arthritis might reflect specific pathogenetic routes in these inflammatory joint diseases (55).

CONCLUSION

YKL-40 is expressed and secreted by inflammatory cells and arthritic chondrocytes. Significantly increased expression of both YKL-40 mRNA and protein in human tissues is found in pathological conditions with acute or chronic inflammation. The relationship between the concentration of YKL-40 with conventional parameters, proinflammatory cytokines and sonographic examinations indicates that YKL-40 is associated with progression of inflammatory processes. Based on the recently published information about YKL-40 in inflammatory joint processes and our own studies, we could conclude that the YKL-40 might be a useful biomarker for diagnosis and monitoring of disease activity in rheumatic joint arthritis.

The complete prognostic value of YKL-40 in clinical practice remains to be elucidated.

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